expressed by a developing chick, 63 directionally cloned cDNA libraries were derived from 21 tissues, ranging from early whole embryos (E2; Hamburger and Hamilton stage 10) to differentiated adult structures (e.g. brain and limb). Each of the 350 000 clones generated were sequenced at the 5′ end, and contaminating vector and bacterial sequences were removed before submission to the database. The ESTs are ordered according to the developmental stage or tissue from which they were isolated to facilitate global gene expression analysis, and they can be searched using a BLASTN interface or by keyword (e.g. expressed in 'heart'). Perhaps most importantly, Thomas Weaver (Babraham Institute, Cambridge, UK) said that any chick EST clone could be obtained for a nominal charge of ~£18.00 (see http://www.hgmp.mrc.ac.uk or ARK genomics [http://www.ARK-Genomics.org] for further information).

Preliminary investigation revealed that ~49% of the ESTs had no significant matches when translated and compared with protein databases (i.e. BLASTX), indicating that they could be novel genes. In conjunction with other data, it now appears that the chick genome might have between 25 000 and 30 000 genes spread across 78 chromosomes (2n), although some investigators have put this as high as 60 000 genes in total, which is consistent with recent higher estimations of human gene number. These studies represent a significant advance in the field of chick genomics and open several avenues of biological research that were unavailable previously.

And your genome for free...

As if the arrival of large-scale expression data was not enough for chick biologists, Bin Liu (Beijing Genomics Institute, Chinese Academy of Sciences, Beijing, China) set out a detailed proposal for the sequencing of the

Fig. 1. Green-fluorescent protein (GFP)-labeled granule cell precursors migrating dorsoventrally from the rhombic lip of rhombomere 1 (left) in tissue from a chick embryo. Such techniques are facilitated by the ease with which the chick embryo can be manipulated microsurgically and vitally labeled. Scale bar: 10 μm. Image courtesy of Jonathan Gilthorpe and Richard J. Wingate.

1.2 billion bases that comprise the chick genome (the human genome has 3.2 billion). Using the sequencing infrastructure already present in China/UK and methods refined during the assembly of the Human Genome Project and the indica rice draft genome sequence announced by BGI last October, a shotgun-sequencing strategy was proposed. This will provide at least four to six times coverage of each sequence, followed by ordered assembly of the sequences (i.e. to align overlapping sequences) and genetic mapping to the appropriate chromosome. In all, Liu anticipated that this would require ~10 million sequence reads and take only six months or less, if the finances could be appropriated. To reinforce these predictions, he intimated that the first read of the genome was all but complete!

The imminence of a completed genome was reinforced by Martien Groenen (Wageningen University, The Netherlands), who described the construction of a comprehensive chick genome bacterial artificial chromosome (BAC) contig map consisting of 50 000 clones, and its integration into physical, cytogenetic and genetic maps. Preliminary examination of the data revealed a high degree of synteny (i.e. homologous segments composed of two or more pairs of homologous genes located on the same chromosome) between chick and human chromosomes (~150–200 large blocks of similarity), giving clues to the evolutionary relationships between the species.

Perspectives

It is clear that significant process has been made in the chick to bridge the gap between cellular biology and the underlying genetic regulation. The EST project, in combination with other large-scale sequencing projects proposed for the chick (e.g. BAC construction and analysis), leads the way for functional genomics to expand and complement the existing advantages of the chick. Indeed, it is rumored that a deal was struck at this meeting to complete the Chick Genome Project, which would secure the chick as a viable biological model.

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Evolutionary genomics: molecular evolution at the genomic scale

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The Symposium on Evolutionary Genomics was held in Atami, Japan, from 4 to 6 November 2001. Meeting website: http://spinner.lab.nig.ac.jp/evolutionary_genomics/.

We now have complete genome sequences of ~70 bacterial species, and the genomes of five eukaryote multicellular species are either complete or almost complete. So we need a unifying view of this diverse world of genomic data. The answer is simple: evolution! Hence, this symposium on Evolutionary Genomics, subtitled 'New Paradigm of Biology in the 21st Century'. This report mentions only a few of the

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highlights of this diverse and exciting meeting.

Large numbers of pseudogenes in *Escherichia coli* genome?

Keiichi Homma and Ken Nishikawa (National Institute of Genetics, Mishima, Japan) examined the predicted 3D structure of many proteins coded in the *E. coli* genome using GTOP (http://spock.genes.nig.ac.jp/%7Egenome/gtop.html). He focused his search on open reading frames (ORFs) that have atypical predicted protein structures compared with their orthologs in other bacterial species. If such an ORF is found, it is likely to be a pseudogene. Pseudogenes are thought to be rare in free-living bacteria such as *E. coli*, because, unless they are maintained by natural selection, genes should be eliminated from the genome. After eliminating possible sequencing errors or variations caused by recent mutations, Homma found ~100 pseudogene candidates. This is quite surprising, as only obligate parasitic bacteria were thought to have substantial numbers of pseudogenes in their genomes. If the rate of generating pseudogenes is higher than expected, then a substantial number of pseudogenes will accumulate before they are eliminated through purifying selection.

Comparison of closely related organisms for study of developmental evolution

Comparison of closely related organisms should help dissect the detailed evolutionary steps that caused developmental differentiation of two species. Yoshiyuki Yamamoto of William Jeffery's lab (Dept of Biology, University of Maryland, USA) has been studying evolutionary mechanisms of eye degeneration using two forms of the Mexican freshwater fish species *Astyanax mexicanus*: a surface-dwelling form that has eyes and an eyeless cave-dwelling form (cavefish). There are about 30 cavefish populations with eyeless phenotypes, and their molecular phylogenetic tree suggests that eyelessness has arisen independently several times from the ancestral surface fish. Although a cavefish embryo initially forms small eye primordia, this undergoes apoptosis of the lens, arrested growth and eventually eye degeneration. Transplant of the surface fish lens into the cavefish optic cup can restore eye formation [1]. Yamamoto *et al.* looked for genes that might be responsible for cavefish eye degeneration, and compared the expression patterns of 30 candidate genes in surface fish and cavefish embryos. Only a few genes had modified expression patterns, including *Pax6* and *Pax2*, which are known to be involved in development of various parts of the eye. Interestingly, the sonic hedgehog (*Shh*) gene, which establishes the embryonic midline, also showed a different expression pattern. Jeffreys *et al.* are now doing various experiments to elucidate why *Shh* is associated with eye degeneration.

Co-evolution of interacting proteins

Co-evolution of two genes in the same genome is evidence of gene interaction. Mammalian pituitary growth hormone (GH) gene usually evolves slowly (3.7×10^{-9} substitutions/site/year), but the primate GH gene evolved three times more rapidly, and accumulated more than 60 amino acid substitutions from the common ancestor of all eutherian species. What caused this rapid evolution of primate GH? Soojin Yi and Wen-Hsiung Li (Dept of Ecology and Evolution, University of Chicago, USA) studied this problem. Interestingly, even after accumulating so many amino acid changes, human GH is physiologically effective when administered to other mammalian species. However, human and rhesus macaque growth hormone receptor (GHR) responds only to primate GH. Both physiological and molecular evolutionary analysis suggested that this specificity of primate GHR is largely due to the change of two amino acids [2]. Yi and colleagues also found that the New World Monkey GHR had intermediate amino acid pattern between human–rhesus and other mammals. This GHR can respond physiologically to human GH, but does not exhibit the same specificity as hominoid and Old World Monkey receptors.

Comparative genomics of human and apes

Comparison of hominoid genomes will provide clues to the genetic changes that made us 'human'. As the human genome sequencing effort nears completion, it is now time to start sequencing the ape genomes, particularly that of chimpanzee (*Pan troglodytes*), the most closely related organism to human. Yoshiyuki Sakaki's group (RIKEN Genomic Sciences Center, Yokohama, Japan) has constructed the first map of the chimpanzee genome [3]. They first created a chimpanzee bacterial artificial chromosome (BAC) library, then sequenced both ends (500–1000 bp) of more than 64 000 clones from the library and another library developed by Kazutoyo Osoegawa and Peter de Jong. The 114 000 BAC end sequences were compared against the human genome draft sequences using the BLAST program. Although coverage of the chimpanzee genome using this method is only ~50%, it will increase as the human genome sequences are completed. This BAC map will eventually allow substitution of current 'wet' screening techniques, such as hybridization and PCR, for *in silico* screening.

An international consortium for sequencing chimpanzee chromosome 22, corresponding to human chromosome 21, is now established, including institutions in Shanghai, Berlin, Daejeon, Mishima, and Taipei, and we are already using this BAC map. However, to examine human-specific genes and expression patterns, we need sequence data from further outgroup species, such as gorilla or orangutan. So, we are also beginning to sequence gorilla and orangutan in our Silver Project (http://sayer.lab.nig.ac.jp/~silver/).

No biologist can argue against the view that all organisms are products of evolution. But biologists who use only model organisms seem to sometimes forget or neglect the outcome of this process, the evolutionary history of organisms. Fortunately, genomics deals with many organisms and importance of 'history' is well appreciated. To quote Hitoshi Kihara, who initiated 'genome analysis' using wheat in 1920s:

> The history of the earth is recorded
> in the layers of its crust;
> The history of all organisms is
> inscribed in the chromosomes.

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